Biosynthesis of Silver nanoparticles (AgNPs) and sustainable management of Mung bean yellow mosaic virus (MYMV) disease.

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INTRODUCTION

Nanotechnology is a science that deals with the production and stabilization of nanoparticles. It is the engineering of functional systems at the molecular scale. In the recent time, Nanoscience and Nanotechnology are gaining interest and attention due to wide range of applications. It is becoming one of the fast growing field. The nanosized materials and structures usually ranging from 1 to 100 nanometers (nm) has various applications and is an emerging area of nanoscience and nanotechnology. Various nanoparticles of silver, gold and copper have been synthesized by using plant, yeast, fungi, bacteria. Among them silver nanoparticles are of great importance because of its good conductivity, stability and antimicrobial activity. In the field of biology and medicine silver nanoparticles play a significant role. Biological method, thermal decomposition of silver compounds (Plante *et al*., 2010), radiation directed (Li *et al*., 2011), microwave directed (Nadagouda *et al*., 2011) and sonochemical methods are used for the synthesis of nanoparticles.

The biological activity of silver nanoparticles depends on various factors such as size, shape, particle composition, particle morphology, capping, agglomeration, etc. (Carlson *et al.,* 2008). Nanoparticles that are synthesized by using biological method are known as biogenic nanoparticles. It is the most reliable method for the biosynthesis of AgNPs as it is safe, cost-effective and eco-friendly and it does not use any chemical compound that is why it is also known as green synthesis. The biological methods of nanoparticles synthesis using biological resources including bacteria, yeast, fungi (Ahmad *et al*., 2002, Ahmad *et al*.,2003, Shahverdi *et al*., 2007, Bharde *et al.,* 2008) and plants (Lee *et al*., 2010 and Torresdey *et al*., 2003) are reported as clean, innocuous and economically satisfactory routes. The biogenic synthesis of nanoparticles opens a new possibility of conveniently synthesizing pure metallic nanoparticles using natural products (Manisha *et al*., 2013). It is well known that silver has strong toxicity effect against wide range of microorganism and silver nanoparticles showed to be promising antimicrobial materials (Jo DH *et al.*, 2015 and Jain e*t al*.,2009). The applications of silver nanoparticles greatly depend on their size. HIV-1 infection is controllable by silver nanoparticles by interaction of nanoparticles (1-10 nm) with HIV-1 virus and preferentially bind to gp120 glycoprotein. (Kim *et al.*,2007). AgNPs and ions known to possess intrinsic cytotoxic action

(Sombaty *et al.*,2013). Antimicrobial activity of AgNPs have been well established and they possess anti-inflammatory, anti-viral, anti-platelet and anti-fungal activity (Kalishwaralal *et al.,* 2009).

Silver nanoparticles have been exploited in medicine for antibacterial, antifungal, antiviral and anti-inflammatory therapy in human system but its application in agricultural system against plant viruses has not been explored. Plant viruses are known to cause many important plant diseases that results in serious losses in crop yield and quality in all parts of the world (Gergerich and Dolja 2006). Out of 15% of the global food production is lost due to plant diseases, among them virus diseases account for 47% of this loss (Anderson *et al*., 2004; Popp and Hantos 2011; Boualem *et al*., 2016).

There are virtually no antiviral compound available to cure plants with viral diseases (Song 2010). Most management strategies to control diseases caused by plant viruses are directed at preventing infection of the plant or based on destroying the affected plant. Hence in the present study we will attempt an alternative economical and sustainable strategy to control plant virus using silver nanoparticles as antiviral solution in the form of liquid that will prevent virus in plants.

In this regard, we will make an attempt for synthesis of AgNPs from the mushrooms and their characterization by UV-Visible Spectrophotometer, Transmission Electron Microscopy (TEM) and Fourier Infrared Spectroscopy (FT-IR), and check the antiviral activity of silver nanoparticles against selected plant virus i.e. MYMV

BIOSYNTHESIS OF NANOPARTICLES

There are two method for the synthesis of nanoparticles:

1) Top to Bottom
2) Bottom to Top

Two main approaches are used in nanotechnology. In the "bottom-up" approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition. In the "top-down" approach, nano-objects are made from larger entities without atomic-level control (Rodgers, 2006).

In my research work i will use Green synthesis method of nanoparticle synthesis which is a type of Bottom to Top method. We will use mushroom extract which will act as reducing and capping agent for nanoparticle synthesis. It is an inexpensive, environment friendly and toxic free approach.

USE OF MUSHROOM TO SYNTHESIZE NANOPARTICLES

For the biological synthesis of nanoparticles many microorganisms (like bacteria, fungi, etc.), biochemical components (like enzyme, polysaccharide etc.) and plants are being used. (Mukherjee *et al.*, 2001; Singh *et al.*, 2016). However, the use of microorganisms especially the fungi is potentially exciting since they secrete plenty of enzymes. This property of fungi has made them an ideal biological candidate for the synthesis of various metal nanoparticles (Dhillon *et al.*, 2012). Comparatively, the higher fungi such as mushrooms in this regard have not received the much attention. Mushrooms are the fleshy fruiting bodies of the Basidiomycetes fungi, typically found above ground in soil, rotten woods or trees. Several reports have been made of their distinctive biological properties such as antibacterial, antioxidant, antiinflammatory, anticancer, antitumor, cytotoxic, anti-HIV, antidiabetic, hepatoprotective and others (Kao *et al.*, 2013). Therefore, in the present study ***Pleurotus ostreatus, Pleurotus djamor, Pleurotus pulmonarius*** important medicinal mushrooms will be explored for the synthesis of silver nanoparticles. These mushrooms are known to contain some favourable compound that can accumulate large amounts of reduced nanoparticles (Arun *et al.*, 2014). Hence, biosynthesis of Ag-NPs by mushrooms will be more safe and eco-friendly. Here, we will demonstrate the biosynthesis of Ag-NPs using medicinal mushrooms, best among them will be selected for screening and study its antiviral potential against plant virus i.e. Mung bean virus

Characteristics of *Pleurotus* spp. of Mushroom:

Classification of *Pleurotus*

Kingdom	Fungi
Phylum	Basidiomycota
Class	Agaricomycetes
Order	Agaricomycotina
Family	Pleurotaceae
Genus	*Pleurotus*

Fig*: P. ostreatus* Photo©GaryEmberge

Pleurotus ostreatus, the **oyster mushroom**, is a common edible mushroom. It is also used industrially for mycoremediation purposes (Kulshreshtha *et al*.,2013).The oyster mushroom is one of the more commonly sought wild mushrooms, though it can also be cultivated on straw and other media. It has the bittersweet aroma of benzaldehyde (which is also characteristic of bitter almonds). It is rich in protein, vitamin C and potassium. It also produces a compound Lovasatin which is known to reduce cholesterol. It is known for anticancer, immunomodulatory, antiviral, antibiotic anti-inflammatory and cholesterol-lowering activities worldwide (Bobek 1991, Shamtsyan *et al*.,2004, Wang *et al.*,2002). The gills of the mushroom are white to cream. The spore print of the mushroom is white to lilac-gray and best viewed on dark background.

Pleurotus djamor, commonly known as the **pink oyster mushroom**. They are found growing on hardwoods in many tropic and subtropic zones throughout the world. Also known as the Flamingo Oyster. They are high in protein and fibre, iron, zinc, potassium, phosphorus and selenium, calcium

folic acid, vitamin B_1, B_3, B_5 and B_{12}, Vit. C and Vit.D.. They have several medicinal benefits like cholesterol lowering, anti- oxidant, anti- cancerous.

Pleurotus pulmonarius, commonly known as the **Indian Oyster**, **Italian Oyster**, **Phoenix Mushroom**, or the **Lung Oyster**, is a mushroom very similar to *Pleurotus ostreatus*, the pearl oyster, but with a few noticeable differences. The caps of *pulmonarius* are much paler and smaller than *ostreatus* and develops more of a stem. *P. pulmonarius* also prefers warmer weather than *ostreatus* and will appear later in the summer. Fruit body are rich in protein, carbohydrates, Na, K, and Ca. It was also contain significant amount of Alkaloids, Tannins and Saponins and is useful in drug synthesis (Paul 2000).

TARGET PATHOGEN

Mung bean yellow mosaic virus (MYMV):

MYMV disease is caused by begomoviruses and is also known as geminiviruses which are the leading and the most significant genus within the family, Geminiviridae. They are plant infecting single-stranded DNA viruses. It is transmitted by vector white fly (*Bemisia tabaci*). MYMV infection lowers the productivity level of crop. (Karthikeyan *et al*.,2014)

Host: MYMV infect mostly dicotyledonous plants like mungbean, urdbean, cow pea and soyabean (Haq *et al.* 2011). *Vigna radiata* (Mung bean) also known as green gram is one of the inexpensive source of vegetable dietary protein in India. It is very sensitive crop to insects, weeds and diseases caused by fungi, virus and bacteria. Among them viruses are most cruicial group of plant pathogen that cause serious diseases and economic losses which decreases the seed yield and quality of crop (Kang *et al.*,2005). Many strategies for management of MYMV disease are there such as planting resistant or tolerant varieties, vector management and modifying the cultural practices of the crop but these are not effective in managing the disease and are lesser supportive for disease development. Many studies have shown that virus infection can be controlled by the use of insecticides and pesticides available in market but they are toxic for humans as well as for animals, some

become concentrated as they spread through food chain. All the resistance found in mung bean appears to be only tolerance against the infection (infection with mild symptoms) rather than complete immunity (Karthikeyan *et al.*, 2015). Therefore, there is need to develop a better management practices. With this background, I will make an attempt for alternative method to control MYMV.

REVIEW OF LITERATURE

We will presumably find ourselves engrossed in the field of nano-biotechnology very soon. It has now become a powerful basic and applied science tool drawing attention of the scientific community towards itself. The conventional methods for the synthesis of nanoparticles using chemical means disturb the eco-system which possess a serious threat to environment and thus novel methods that promotes the ecological safety are to be explored that may include the use of several living systems for nanoparticles synthesis. A step has been taken to synthesize nano-sized particles of interest with a wide range of biological systems from bacteria, plants, fungi, yeast. These biological molecules act as reducing agents and oxidizing agents that are significantly safe over chemicals use thus do not need chemical reagents for synthesis. Taking into account the above aspects, biological resources are the best alternative source rather than all the other means for synthesis of nanoparticles (Riddin *et al.*, 2006 and Narayana *et al.*, 2010). In recent years, biological resources have replaced chemical methods for the synthesis of silver nanoparticles (Bansal *et al*., 2004, 2005; Kumar *et al*., 2007). Mushrooms (macrofungi) are immensely wealthy in proteins and have been studied for their nutrient-richness as food stuffs and for their great medicinal value. Therefore, the biological synthesis of AgNPs from mushroom extracts will provide a novel, simple, rapid, reliable, cost-effective and environmentally friendly approach for metallic nanoparticle synthesis. However, very few reports have demonstrated the potential use of mushrooms for synthesis of metallic nanoparticles and most of the reports available only on their antibacterial

and antimicrobial activity. Some reports of silver nanoparticles as antiviral agents against human virus are available but in the field of plant virus it has not been explored so much.

Following workers are reported to synthesize silver metal based nanoparticles and their antibacterial and antimicrobial activity on the basis of literature survey-

S.No.	Fungus Species	NPs	Size(nm)	Application	Paper	Reference
1.	*Agaricus bisporus*	Silver	8-50	Antimicrobial	Mushrooms (*Agaricus bisporus*) mediated biosynthesis of sliver nanoparticles, characterization and their antimicrobial activity	Narasimha *et al*., 2011
2.	*Agaricus bisporus, Calocybe indica,* (Bar H, 2014) *Pleurotus florida,* and *P. platypus*	Silver	1-5	Antibacterial	Studies on biosynthesis of silver nanoparticles using mushroom and its antibacterial activities	Sujatha *et al*., 2013
3.	*Agaricus bisporus,,Helvella lacunose, Fomes fomentarius, Pleurotus florida. Ganoderma appalanatum*	Silver	20-44	-	Isolation and Screening of Mushrooms for Potent Silver Nanoparticles Production from Bandipora District (Jammu and Kashmir) and their characterization	Manzoor ul haq *et al*., 2014
4.	*Agaricus bisporus*	Silver	30 ±15	Antimicrobial	Synthesis of Silver Nanoparticles from Edible Mushroom and Its Antimicrobial Activity against Human Pathogens	Sudhakar *et al*.,2014
5.	*Schizophyllum commune*	Silver	-	Biomedical	Green synthesis of silver nanoparticles using the mushroom	Ganesan *et al*., 2015

					fungus *Schizophyllum commune* and its biomedical applications	
6.	*Pleurotus citrinopileatus*	Silver	6-10	Antibacterial	Green Synthesis of Silver Nanoparticles using Pleurotus and its Bactericidal Activity	Maurya *et al*., 2016
7.	*Ganoderma Lucidum*	Silver	10-30	Antibacterial and antioxidant	Biosynthesis of Silver Nanoparticles Using *Ganoderma Lucidum* and Assessment of Antioxidant and Antibacterial Activity	Poudel *et al*., 2017
8.	*Pleurotus ostreatus*	Silver	Less than 40	Antibacterial	Green synthesis of silver nanoparticles using tree oyster mushroom *Pleurotus ostreatus* and its inhibitory activity against pathogenic bacteria	Bahrani *et al.*, 2017
9.	*Pleurotus florida*	Silver	-	Antimicrobial	Synthesis of Silver Nanoparticles from *Pleurotus florida*, Characterization and Analysis of their Antimicrobial Activity	Kaur *et al.*, 2018
10.	*Ganoderma sessiliforme*	Silver	-	Antimicrobial	Silver Nanoparticles Synthesized Using Wild Mushroom Show Potential Antimicrobial Activities against Food Borne Pathogens	Mohanta *et al.*, 2018

From best of my knowledge reports on antibacterial and antifungal activity of silver nanoparticles synthesized from *Pluerotus* species are available but no reports are available for their antiviral activity against MYMV.

OBJECTIVES

1. Cultivation of mushroom and their extract preparation for biosynthesis of silver nanoparticles.
2. Screening of *Pleurotus* species for production of silver nanoparticles.
3. Characterisation of silver nanoparticles through UV-Vis spectroscopy, XRD, FTIR, EDX and SEM.
4. To test the antiviral activity of prepared silver nanoparticles from selected *Pluerotus* spp. Against plant virus i.e, MYMV.

METHODOLOGY

1. **Cultivation of mushroom**: The mother culture for spawn production will be brought from ICAR Solang. Species of Pluerotus mushroom will be grown in microbiology lab according to Singh et al.,2012.

2. **Preparation of mushroom extract –**

Fruiting body

⇩

Wash with autoclave water

⇩

Chop the fruiting body into small pieces with sterilized knife

⇩

Crush with liquid Nitrogen in mortar

⇩

Transfer the sample into flask containing distilled water

⇩

Stir the mixture 24 hr at 200 rpm

⇩

Next day filter the extract and store it at 4ºC for further use

3. **Synthesis and screening of silver nanoparticles** - 0.01 M, 0.001 M and 0.002 M concentrations of silver nitrate (AgNO3) respectively would be prepared in distill water and will be used for synthesis of silver nanoparticles. Mushroom extract will be added to each concentration of aqueous solution of silver nitrate solution for reduction of Ag+ ions into Ag and will be observed for production of AgNPs at different time intervals over a period of 24 hours (Bankara *et al*. 2010, Banerjee et al. 2011 and Khadri *et al*. 2013). The biochemical reaction of mushroom extract with AgNO3 leads to the formation of AgNPs by following reaction showing mechanism of biosynthesis of silver nanoparticles:

   Reducing agent + Silver Nitrate ⟹ Silver NPs

4. **Characterisation of silver nanoparticles by UV-Visible spectroscopy, XRD, FTIR, EDX and SEM–** The reduction of pure Ag+ ions will be monitored by measuring the UV-Vis spectrum of the reaction mixture after diluting a small amount of sample into distilled water (Shankar et al. 2003). Purified silver particles by repeated centrifugation will be collected for the analysis of their sturcture and composition by XRD. FTIR analysis will be done to confirm that bioreduction of Ag+ ions to silver nanoparticles is due to the reduction by capping material of mushroom extract (Bansal *et al*. 2005 , Huang et al. 2007 and Sanghi *et*

al. 2009) . EDX will confirm the presence of elemental silver and shape of the nanoparticle. SEM will be used for detection of the size of the nanoparticle. (Bar *et al.* 2009).

5. **Antiviral assay**: Virus inoculum and healthy seeds will be obtained from Jaipur National University. These will be sown in clay pots filled with soil compost (3:1) mixture and transferred to an insect free net house. The experiment will be perform with minimum three replicates. The leaves in net house will be treated with different concentrations of AgNPs solution and virus inoculums. TEM will be done of AgNPs with interaction with virus inoculum. This will show that AgNPs bind to coat protein virus particles. (Dougdoug *et al* .2018 and Jain and Kothari 2014)

SIGNIFICANCE

Green synthesis of nanoparticles is the hottest topic being studied in the last two decades. This production of metallic nanoparticles is induced by the metabolites and enzymes present in plants, fungi, bacteria. Thus, offering the large scale production of nanoparticles in an eco-friendly manner. Various diseases can be diagnosed and treated with nanoparticles with great potency and also prevents side effects up to a large extent. Silver nanoparticles bind to virus particles and inactivates the virus by inhibiting virus replication in host plant. Mung bean yellow mosaic virus (MYMV) disease is one of the most important and destructive diseases of mung bean. It is caused by Mung bean yellow mosaic geminivirus belonging to the genus, begomovirus of the family, Geminiviridae. The virus is transmitted through whitefly vector in a persistent manner. The economic losses due to this virus account up to 85% in mung bean which is spreading faster towards newer areas. In fact, MYMV is a threat to mung bean production in the country and the mung crops of Dayalbagh are also known to be affected by MYMV. In view of the economic importance of MYMV disease it is important to make eco-friendly measures to inhibit the virus infection. Therefore, the aim of my research is to examine whether silver nanoparticles treatment can induce signs of resistance against BYMV in infected *Vigna radiate*.

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